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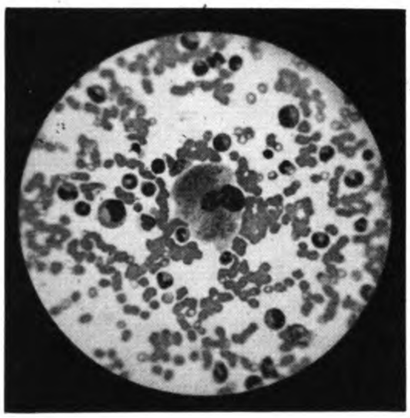
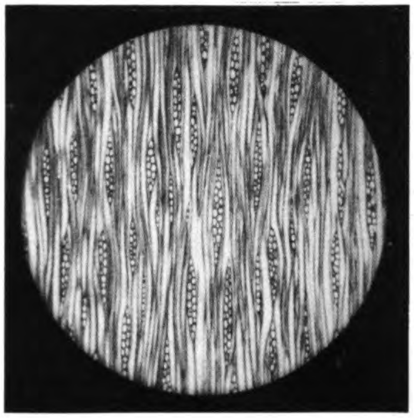
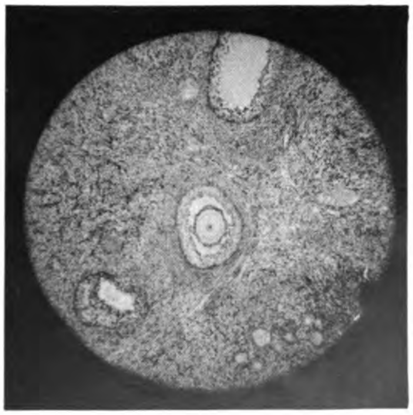




PHOTOMICROGRAPHY WITH **SIMPLE CAMERAS**



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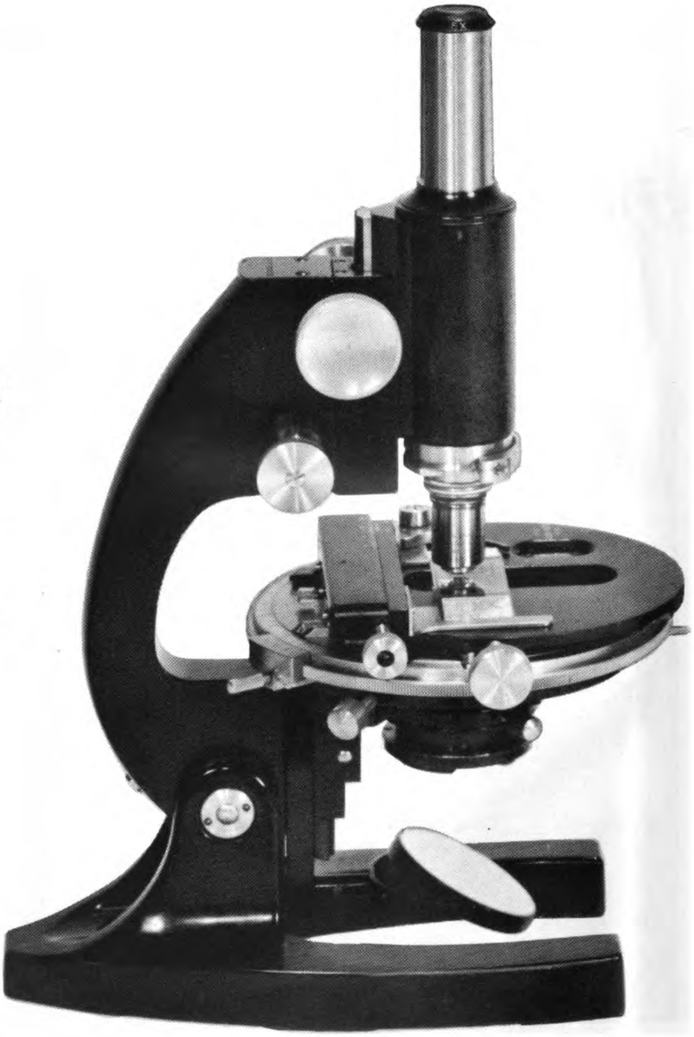
PHOTOMICROGRAPHY WITH SIMPLE CAMERAS

COVER

TOP: Megakaryocyte in normal bone marrow
15X Hyperplane ocular
50X Oil immersion objective

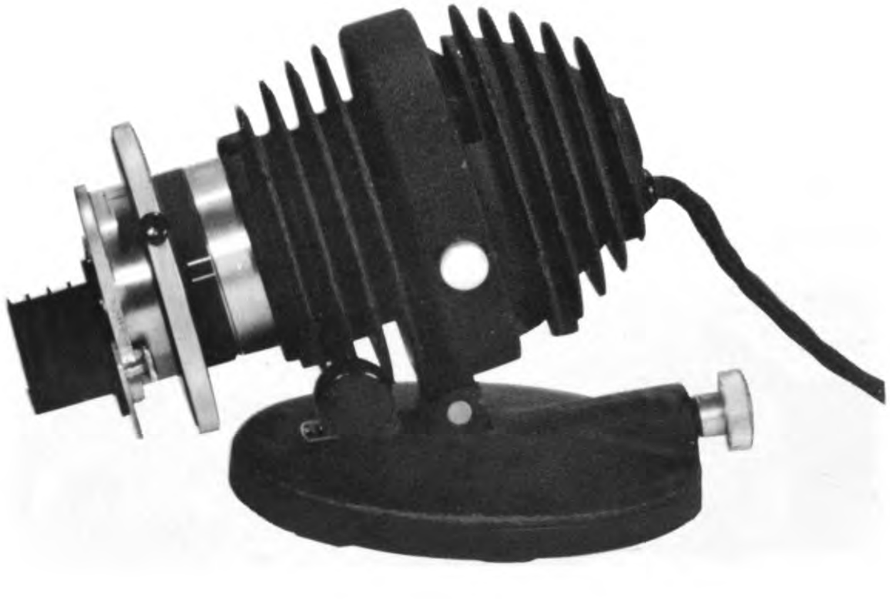
MIDDLE: Hickory (X-section)
15X Hyperplane ocular
10X Objective

BOTTOM: Cat ovary showing Graafian follicle
15X Hyperplane ocular
10X Objective



- Anyone can take a simple, inexpensive camera and make reasonably good photographs, or snapshots. This is evident since a good many thousands of such cameras are in daily use throughout the world. The camera is pointed at the subject and the shutter snapped. No adjustments for exposure or distance are necessary. The film is delivered to a photofinisher who develops it and produces finished prints.

Occasionally, the owner of such a camera has the opportunity of using a microscope either as a hobby or, possibly, as a student or a teacher of science. The microscope presents a new world of subjects for visual study and also for photography. It is only natural, then, when an interesting specimen



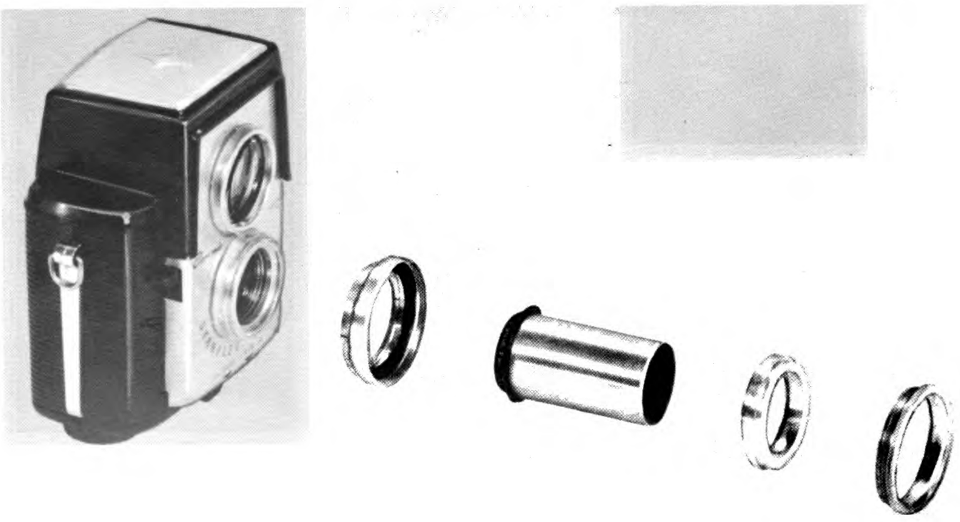
is seen through the microscope, to want to photograph it. First attempts to couple an inexpensive camera to a microscope and make such pictures may produce disappointing results unless certain simple precautions are observed. It's virtually impossible to just hold the camera over the microscope eyepiece, snap the shutter, and expect to get superlative results. However, it is possible with a little care, to properly adapt such a camera to a microscope and to make reasonably good photographs. (The accepted term to describe photographs made through a microscope is "photomicrographs.")

Adapting the camera to the microscope is relatively easy, but some thought should be given to the other problems involved, such as the correct eyepiece for the microscope, the illumination, the film to be used, and the exposure of the film. These are some of the factors that contribute to the quality of the photomicrograph. The photomicrographs illustrated in this pamphlet were made using a Brownie Starflex Camera over a conventional biological microscope.

The Microscope Adapter

An adapter for holding the camera in position over a standard microscope can be assembled simply and at low cost. If one of the Brownie Star Cameras (for example, the Brownie Starlet, the Starflex, or the Starflash) is to be used, all that is needed is a Series 5 adapter ring, a Series 5 retaining ring, and a small ring of metal, wood, or plastic to fit inside

Figure 1a Photographic apparatus needed to make pictures through the microscope.



the retaining ring and hold the microscope eyepiece in place. Adapter rings and retaining rings can be purchased from any camera store. The inner ring must be constructed. Its inside diameter should be just slightly larger than the diameter of the microscope eyepiece. Figure 1 shows how these parts fit the eyepiece and how the assembly fits the camera.

The Series 5 adapter ring in 28.5mm (1 1/8 inch) size fits the Brownie Star Cameras. Other cameras, of course, could also be used in like manner by selection of the correct adapter ring.

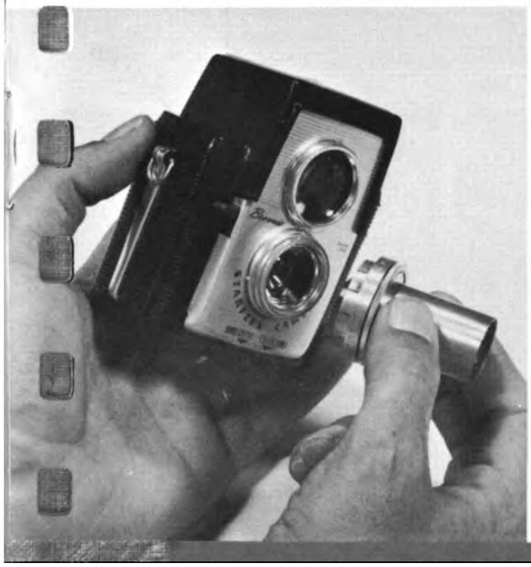
During use, the rings are mounted on the microscope eyepiece (also called the "ocular"). The microscope is focused visually as in normal usage. The ocular, with rings attached, is then removed from the microscope and fastened with the adapter ring to the camera lens barrel. The ocular, then, with camera attached, is replaced in the microscope and the shutter actuated to make the exposure. This action of removing the ocular, replacing it, and snapping the shutter must be done very carefully to avoid upsetting the critical focus of the microscope.

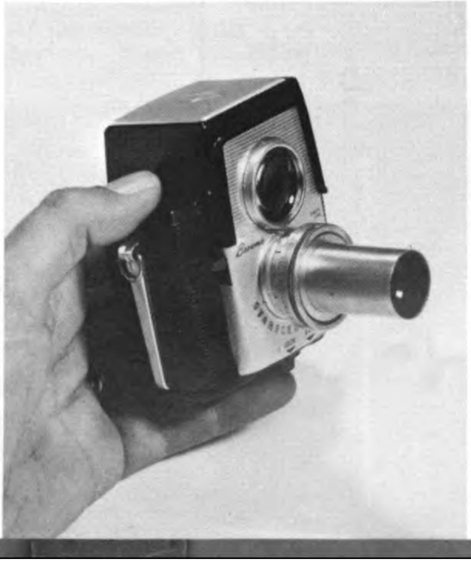
In order to obtain the largest possible field size on the film, the choice of microscope ocular should be carefully considered. The position of the ocular "eyepoint" in the camera is quite important in this respect.

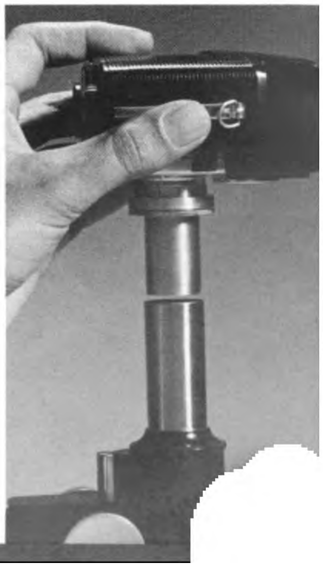
Figure 1b The rings-ocular assembly about to be placed on the camera taking-lens bezel.

Figure 1c The rings-ocular assembly properly positioned on the camera.

Figure 1d Carefully slip the whole business into the microscope tube.







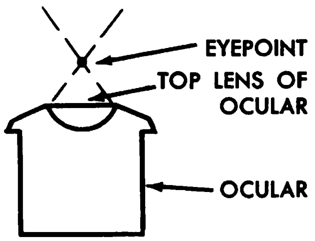


Figure 2

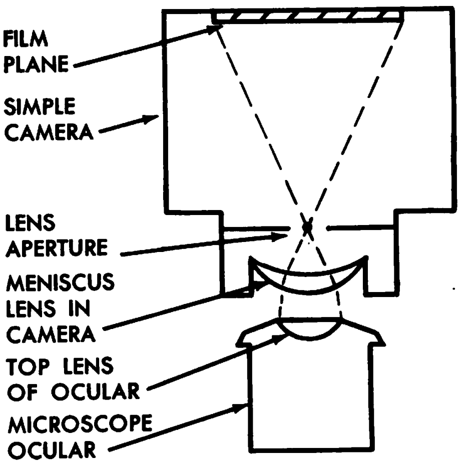


Figure 3

Selection of Ocular

As the light rays emerge from the ocular of the microscope, they converge to a point called the eyepoint (Figure 2), and then diverge to form an image of the micro specimen at a finite distance above the ocular. This eyepoint is the position which the human eye naturally seeks in order to see the full field in the microscope. The position of the eyepoint can occur at different distances above the ocular depending upon the type of ocular in use. With one group of oculars, the distance of the eyepoint varied from 6 to 20mm above the top lenses of the oculars. The distances for these oculars are shown in the table below.

TYPE OF OCULAR	DISTANCE – EYEPOINT TO OCULAR
10X HYPERPLANE	6mm
15X HYPERPLANE	14mm
20X HYPERPLANE	8mm
10X WIDE FIELD	11mm
15X WIDE FIELD	11mm
20X WIDE FIELD	7mm
10X HUYGHENIAN	10mm
12.5X COMPENSATING	20mm
25X COMPENSATING	7mm

The position of the eyepoint can be determined easily by holding a piece of white paper over the ocular and moving it up and down. The position where the circle of light is smallest is the eyepoint.

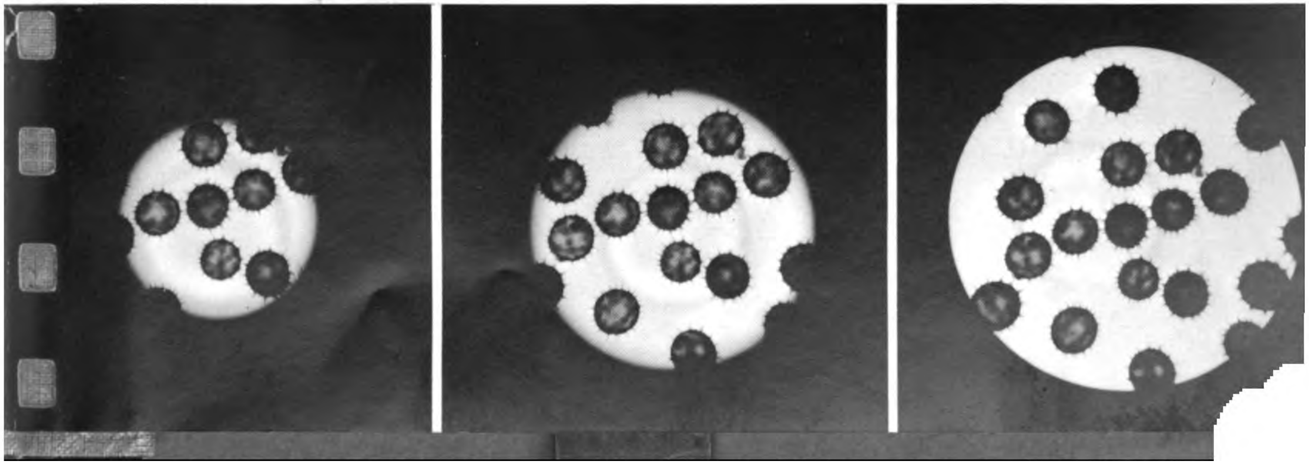
The aperture in a fixed or universal-focus camera, such as the Star Cameras already mentioned, is usually very small and is not variable as it is in more expensive cameras. The aperture allows only enough light to pass through the lens to provide correct exposure in conjunction with the fixed shutter speed and a specified film. This aperture in Star Cameras is about 10mm behind the front surface of the camera lens. The distance may vary with other cameras of the same general type. If more than one aperture is available, the largest should be utilized. Use the "Color" setting on Star Cameras. When such a camera is placed over a microscope, and when the ocular for the microscope is properly selected, the eyepoint of the ocular will occur at the position of the aperture so that the largest image field will show on the film (Figure 3). If the eyepoint is much in front of or behind the aperture, the image field will be reduced in size, or "vignetted."

The photomicrographs in Figure 4 show the effects produced when the eyepoint occurs at three different positions relative to the camera aperture. The smaller the aperture in the camera, the more precisely must the eyepoint coincide with the aperture. Cameras with large apertures can be used in photomicrography with much less attention to the position of the eyepoint.

The effect of eyepoint position relative to aperture can be demonstrated easily by holding a card containing a small hole (about 5mm diameter) over the microscope ocular.

Figure 4

Photomicrographs of hibiscus pollen made with equipment shown in this pamphlet. Microscope optics: 20X hyperplane ocular; 10X objective. Kodak Wratten Filter pack: G (No. 15) plus 0.30 neutral density. Kodak Verichrome Pan Film was developed in Kodak Microdol-X Developer. Left: Eyepoint is in front of aperture. Center: Eyepoint is behind aperture. Right: No vignetting – eyepoint falls at lens aperture.



Then, if a piece of white paper is held over the card and the card is moved up or down so that light from the ocular passes through the hole, the vignetting effect of the hole will be evident on the paper when it is above or below the eyepoint.

If a piece of paper is held over the ocular at the eyepoint, the eyepoint distance can be measured quite accurately. When a particular camera is to be used with the microscope, it is a simple matter to measure or estimate the distance of the aperture behind the front surface of the camera lens. Add to this distance the separation between the top surface of the ocular and the camera lens when the camera is fitted over the microscope. If the sum equals the eyepoint distance, then an efficient arrangement has been obtained. If it is much greater or smaller than the eyepoint distance, then a different ocular should be selected (if possible) in order to obtain a larger image size in the camera. Remember, for best efficiency, an ocular should be selected whose eyepoint occurs at the position of the camera aperture. Of course, everyone does not have a complete selection of oculars, but, when more than just one is available, the best of those available should be used. At the time this pamphlet was prepared, for example, there were three oculars among those available which were tested and which proved efficient with Star Cameras. They were the 10X Wide Field, the 15X Wide Field, and the 15X Hyperplane eyepieces. The 15X Wide Field gave the largest field size on film. Other oculars had either too short or too long an eyepoint distance.

The Star Cameras use size 127 film, which is relatively small. Cameras of similar type which use the larger size 120 or size 620 film have lenses of slightly longer focal length, and, consequently, the distance of the aperture behind the lens may be greater than in the Star Cameras. This means that oculars having longer eyepoint distance would probably be more appropriate. It is possible, therefore, to efficiently adapt most any of the simple cameras to a microscope if proper relationship between ocular eyepoint and camera aperture is found.

Since fixed-focus cameras usually have only one shutter speed, about 1/40 second, a problem is evident regarding correct exposure. If a very bright light source, correctly adjusted, is used with the microscope, the above shutter speed will prove adequate at high magnification with a moderately fast film, such as Kodak Verichrome Pan. When the light source is less bright, it may be necessary to use a faster film, such as Kodak Tri-X Pan. Low and medium power present much brighter images than are evident at high magnification. The same exposure, however, can be used for all magnification ranges if it is correct for high magnification; since overexposure at the lower range will still be within the film latitude. A negative made at low power, then, may be dense appearing but can produce a reasonable print.

When photomicrographs are to be made in color the problem is more acute; particularly if the film is the transparency type, such as Kodak Ektachrome Film. Reversal color films have very short exposure latitude. This means that if exposure is correct at one magnification it will probably be too great at lower power and produce overexposure. At higher magnification the image will be less bright and underexposure will result.

Light sources normally used in microscopy produce low brightness images at high magnification. The image is bright enough to see in the microscope but may not be bright enough to record efficiently on film, especially color film. The brightness may, however, be enough at the lower powers. The lower magnification range is more generally used in amateur microscopy so that exposures will be adequate. Exposure can only be determined by experimentation since, due to the wide variety of light sources and other equipment, it is difficult to make a specific recommendation.

If exposure is correct at very high magnification with color film, the brightness of the illumination can be modified with neutral density filters so that it will be correct at lower magnifications. For example, if a high power oil immersion lens is used to produce very high magnification and exposure is correct for Ektachrome Film, a neutral density of 0.1-0.2 in the illumination will reduce the brightness so that exposure will be approximately correct when the next lower power high dry objective is used. The brightness at even lower magnification will require a neutral density of 0.3-0.4 to produce efficient exposure.

A recommended experiment is to make a series of exposures at the various magnifications available. If none are correct, all may be underexposed and a brighter light source is probably indicated. The possibility of overexposure at all magnifications is practically negligible. If one exposure is correct, then the illumination can be modified with neutral density filters so that correct exposure can be obtained at lower magnifications. This same procedure can also be used with black-and-white films to produce correctly exposed negatives.

Filters

Generally in photomicrography a light green filter is used with black-and-white films. Most often specimens are stained for photography, or even for just visual examination, and the purpose of the green filter is to render the different stain colors in tones of gray which are representative of the color brightnesses of the original. Also, a green filter is used to obtain the best possible optical image quality. The specific green filter often is determined by the colors to be photographed and their relative brightnesses. Generally, either the Kodak Wratten Filter No. 13 (X2) or the No. 58 (B) is used. The latter will produce more contrast between reds and the background than will the former.

As mentioned in the previous section, neutral density filters can be used to reduce illumination brightness by definite amounts. Such a filter will not affect the color of the subject since it has no color of its own. A neutral density of 0.3 will transmit 50 per cent of the illumination, and a density of 0.6 will transmit only 25 per cent. Other neutral densities will transmit greater or lesser amounts depending upon specific density value. It should be noted that high neutral densities (1.0 and above) will impart a yellowish tint to color films.

Another type of filter, used in the illumination when color films are to be exposed is called a Light Balancing Filter. The purpose of such a filter is to correct the illumination so that it will be right for the film is use. The Kodak Data Book

"Photography through the Microscope" contains a table which lists the suggested Light Balancing Filters to be used with different light sources and the various color films.

Illumination

In order to obtain a bright, efficient image in the microscope, it is necessary to use a specific type of light source in a system called Köhler illumination. For best results, this procedure should be followed. The details of setting up a light source and microscope are outlined in the Kodak Data Book "Photography through the Microscope" as well as in other books and literature on microscope procedures.

Magnification

When you look into a microscope, you see an image which is considerably larger than the specimen. The degree of such enlargement is called the "magnification" and is determined by multiplying the power of the objective lens by the ocular power. A 10X objective, for example, used with a 10X ocular will provide a magnification of 100X. Thus, when a standard microscope is used, this magnification on film would be reproduced when the film plane is 10 inches from the eyepoint of the ocular. If the film is placed at a lesser distance, the magnification would be decreased proportionately. When the Star Cameras are used with a microscope, the film is only about 2 inches from the ocular eyepoint. This means that 100X magnification then would only be about one-fifth of that in the microscope. In order to reproduce the original magnification, then, it would be necessary to make a 5X enlargement of the photomicrograph when a print is made.

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